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Research paper

Tight junctions and tight junction proteins in mammalian epidermis

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ABSTRACT

Tight junctions (TJ) are barrier forming cell–cell junctions that are found in a variety of cell types and tissues but their existence in mammalian epidermis has been shown only in the last years. A variety of TJ proteins were identified in mammalian epidermis, comprising several members of the claudin family, occludin, and JAM-A as well as ZO-1 and MUPP-1. TJ proteins exhibit complex expression and localization patterns in the epidermis. Nonetheless, even though several TJ proteins are found in various layers, typical TJ structures are only found in the *stratum granulosum*. TJ are important for barrier function of the skin, especially for inside–out barrier. In addition, TJ proteins might also be involved in additional functions in epidermal cells. Localization and expression of TJ proteins are altered in several skin diseases, e.g. psoriasis. Meanwhile several TJ modulators are known from simple epithelia. We discuss their putative usability for drug delivery into and through the skin.

1. Introduction (TJ in simple epithelia and endothelia)

Tight junctions (TJ) are cell–cell junctions that connect neighbouring cells closely. They are very complex structures that are formed by various TJ transmembrane proteins, e.g. the family of claudins (Cldns), occludin and the family of junctional adhesion molecules (JAMs), as well as TJ plaque proteins, e.g. the MAGUK proteins ZO-1, ZO-2, ZO-3, cingulin, symplekin and the cell polarity complex proteins aPKC, Par3 and Par6. TJ structures and/or TJ proteins control the paracellular pathway of molecules ("barrier function") including the paracellular migration of inflammatory cells. Furthermore, they separate the lipids of the apical from the basolateral portions of the plasma membrane which results in the establishment of two different membrane compartments ("fence function"). Consequently, they establish cell polarity for lipids (for reviews see [1–3]). In addition, TJ structures and/or TJ proteins fulfill a variety of further functions, e.g. they are contact sites for molecules of signal transduction pathways and cell surface receptors, e.g. TGFβ receptor, they are involved in cell proliferation and differentiation and they contribute to vesicle transport (for reviews see [1,4]). Precise function of TJ depends on their composition which, in turn, depends on cell type and differentiation as well as on physiological and pathological stimuli. For example, combination and mixing ratio of the proteins of the Cldn family, which comprises 24 members in vertebrates, is, among others, essential for TJ permeability and ion selectivity [5,6].

Several TJ associated proteins are not restricted to TJ but are also found in the cell nucleus, e.g. ZONAB, c-jun and c-fos, which are known to be transcription factors, symplekin, which is associated with the polyadenylation machinery, and ZO-1, ZO-2, and ZO-3 which are known to play scaffolding functions at TJ, whereas their role in the cell nucleus is not completely clear. ZO-1 has also been identified at adherens junctions and gap junctions (for reviews see [1,2,4,7,8]).

Mutations in genes coding for TJ proteins are the origin for several inherited diseases, e.g. NISCH syndrome (neonatal sclerosing cholangitis associated with ichthyosis) which is caused by mutations in the gene coding for Cldn-1, non-syndromic deafness which results from mutations in the gene coding for Cldn-14, and hypomagnesaemia with hypercalciuria and nephrocalcinosis which originates from mutations in the gene coding for Cldn-16/paracellin1 (for reviews see [9,10]). In addition, alterations of TJ proteins and structures are found in several other ailments, e.g. in inflammatory diseases, such as Morbus Crohn and acute lung inflammation [11,12] as well as in various tumors (for review see [13]).

TJ proteins are known to be targets for bacterial, viral and allergic insults. For example, the enterotoxin of *Clostridium perfringens*, which causes severe diarrhoea, binds directly to Cldn-3 and -4 – originally identified as "*Clostridium perfringens* enterotoxin-receptors – and separates Cldn-4 from TJ [14,15]. Still, the importance of binding to Cldn-3 and -4 for the clinical symptoms is not clear, as the NH2-teminal region of the enterotoxin increases membrane permeability by forming small pores [16,17]. Besides direct inter-

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actions, TJ can also be altered secondarily during bacterial infection, e.g. via signal transduction pathways and alterations of the actin filament cytoskeleton (for reviews see [18,19]). Viruses have been described to use TJ proteins as (co)receptors, e.g. JAM-A is used by reovirus and Cldn-1 by hepatitis C virus [20,21]. Allergens with proteolytic activity, e.g. Der p-1, a cystein-protease of the house dust mite, can disrupt TJ and might thereby facilitate the accessibility of epithelia to the allergens and be important for the development of asthma (for reviews see [1,2,18,22]).

2. TJ proteins and structures in the epidermis

The existence of TJ in mammalian epidermis has – after decades of discussion [23–32] – finally been demonstrated in both human and mouse epidermis at the beginning of this century [33–36]. The successful identification of TJ structures is ascribable to the use of antibodies recognizing TJ proteins as markers, allowing a more specific search for the structures. Previous reports questioning the existence of TJ structures mostly did not have the benefit of these antibodies for their studies.

Up to now a variety of TJ proteins have been identified in mammalian epidermis by using specific antibodies, i.e. Cldn-1, Cldn-3 (faintly), Cldn-4, Cldn-5 (faintly), Cldn-7, occludin, JAM-A, cingulin, ZO-1, Mupp-1, and symplekin, in human and additionally Cldn-6, Cldn-11, Cldn-12 and Cldn-18, ZO-2, aPKC, Par3, and Par6 in mouse epidermis (investigation of most of these proteins has not been done as yet in human skin but some of the proteins have already been identified in human cultured keratinocytes) [23,24,34,36–42]. Additional TJ molecules, e.g. Cldn-8 and Cldn-17, have been identified on mRNA level in human keratinocytes. The distribution patterns of the various TJ proteins in the epidermis are diverse. While, e.g. occludin and cingulin are restricted to the *stratum granulosum* (SG), some proteins, e.g. ZO-1 and Cldn-4 are found in several suprabasal layers, and other proteins, e.g. Cldn-1 and MUPP-1 are localized in all epidermal layers (Fig. 1).

Typical TJ structures comparable to those known from simple epithelia, i.e. seen in transmission electron microscopy as small subapical regions of direct contact between the plasma membranes of two adjacent cells without extracellular gaps or intermembranous material (“kissing points” or “sites of fusion”) (Fig. 2) are found in the lateral plasma membranes of the keratinocytes in the SG of human and mouse. This is also the area, where (1) all TJ proteins present in the epidermis colocalize, (2) apparently continuous zonula occludens-like immunostainings in horizontal sections are found [34,43], and (3) the extracellular diffusion of an intradermal injected tracer of 600 Da stops, hinting for functionality of these structures as a barrier (Fig. 1) [35,41,44]. The latter has up to now only been published in mouse skin. The typical TJ structures found in the epidermis are often interspersed with desmosomes [34,35,43]. As several TJ proteins also colocalize in deeper layers of the epidermis, additional TJ-related structures with putatively different barrier properties, might be postulated, but have not been shown as yet. In various stratified epithelia, e.g. bovine gingiva and muzzle epithelium, other structures containing occludin have been identified, including occludens junctions, lamellated junctions (coniunctiones laminosae) and sandwich junctions (iuncturae structae) [43]. The function of these structures is so far unknown.

A network of anastomosing fibrils which is typical for TJ structures in freeze-fracture electron microscopy in simple epithelia and endothelia has been found in vitamin-A or humid milieu treated chicken and mouse epidermis [27,28], cultured human skin keratinocytes [32], and occasionally in human fetal skin [30]. In addition, structures similar to these have been identified at the border between SG and *stratum corneum* (SC) in human epidermis [45]. However, in the SG of adult untreated skin these structures have not been identified as yet. The difficulties in identification may be due to their restricted localization in mammalian skin.

In addition to keratinocytes, Cldn-1 has also been identified in stationary and migratory Langerhans cells [46].

TJ proteins have also been identified in human and mouse hair follicles and in human cutaneous glands [43,47,48]. This argues for a continuous TJ system in the skin, but typical TJ structures or TJ-related structures in skin appendages have not been shown as yet.

3. Dynamics of TJ in the epidermis and in cultured keratinocytes

In cultured keratinocytes it has been shown that the formation of (functional) TJ depends on Ca-concentration. Ca^{2+} induced differentiation of human and mouse keratinocytes is accompanied by a continuous localization of TJ proteins at the cell–cell borders [34,36,38,49,50] and by the establishment of transepithelial resistance (TER), as well as by decreased permeability for molecular tracers, both measures for TJ tightness [38,49,50]. Ca^{2+} depletion results in a loss of TJ proteins from the cell–cell borders and a decrease in TER [50]. Inhibition of aPKC which is associated with TJ as part of the cell polarity complex Par3/Par6/aPKC abolishes the establishment of TER after Ca-induced differentiation of mouse keratinocytes; overexpression of aPKC accelerates TER formation [38].

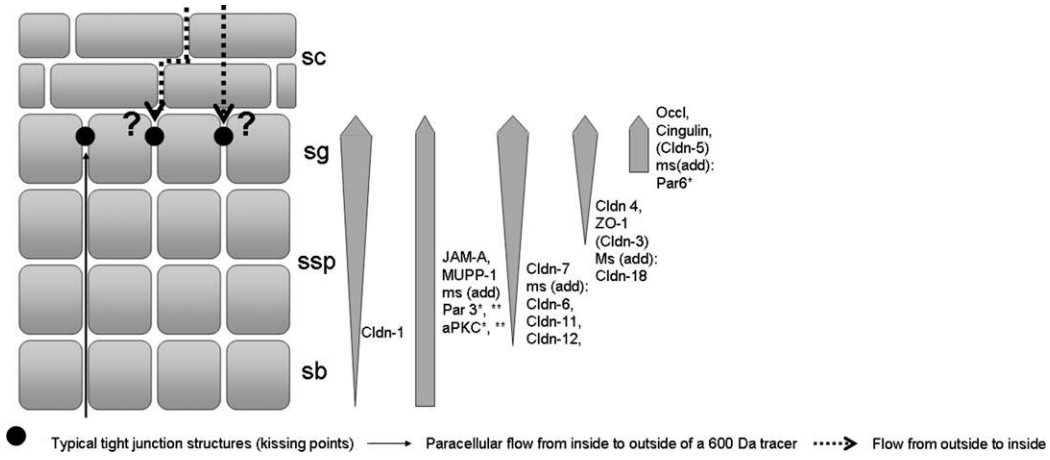

Fig. 1. Schematic drawing of the epidermis showing localization of typical TJ structures as well as of TJ proteins. *Different localizations of various isoforms; **partly in the cytoplasm and at cell–cell borders; sb, stratum basale; ssp, stratum spinosum; sg, stratum granulosum; sc, stratum corneum; ms, mouse, add, additional. Proteins mentioned in brackets have been observed only very faintly.